# The Embryo Takes Its Vitamins

*A burst of recent work suggests that derivatives of vitamin A have a fundamental role in giving shape to the developing mammalian embryo*

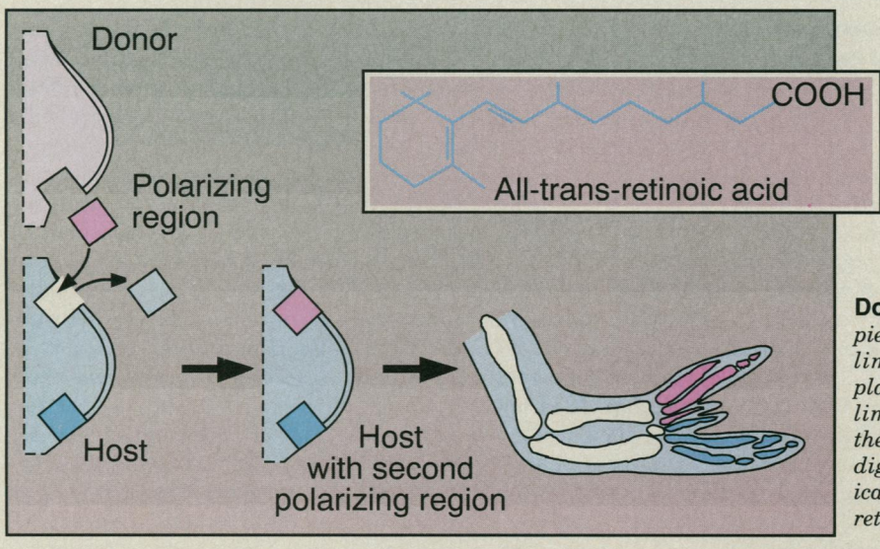
ONE OF THE CENTRAL PROBLEMS IN DEVELopmental biology is understanding how cells in the developing embryo "know" what structures to become. How, for example, does one part of the structure called the limb bud in the chick embryo know it should become digits while another part turns into forelimb? One theory is that molecules called morphogens—literally "shape-givers"—play this specifying role. But what might those form-giving substances be? Recently, work from several key international labs has provided a surprising answer: the retinoids, the group that includes vitamin A and its derivatives.

In the past 3 years retinoids—particularly the one called retinoic acid—have become one of the hottest topics in developmental biology. In a burst of research results, retinoic acid has been established as a good candidate for the fundamental morphogen, its activity has been detected in tissues that have a crucial role in giving the embryo its form, and a set of receptors has been identified that may help to explain how retinoic acid achieves its specific effects. And even more recently the discovery of a new class of receptors hints that other retinoids may also act as morphogens.

These findings are startling because until recently vitamin A has not been thought of in relation to developmental biology at all. Historically, research interest in that substance has centered on its role as a component of light-sensitive pigments in the retina. Because of its biological importance, human beings have evolved carefully regulated mechanisms for the storage and transport of vitamin A and for its conversion to biologically active metabolites. Those mechanisms are the subject of a review by Rune Blomhoff of the University of Oslo and his colleagues on page 399 of this issue of *Science*.

The recent spate of research on retinoic acid as a morphogen was triggered by work in the laboratory of Gregor Eichele at Harvard University Medical School. Eichele, however, was drawing on a classic model in embryology, which holds that morphogens spread across the embryo in a concentration gradient; cells then become differentiated on the basis of how much morphogen they are exposed to.

About 20 years ago that model gained credence as the result of some remarkable experiments by John Saunders and Mary Gasseling, then at Marquette University. When Saunders and Gasseling grafted part of the posterior portion of one chick limb bud onto the anterior portion of another limb bud, they found that the recipient bud developed an extra set of digits, in a mirror image of the first set. The grafted region came to be known as the ZPA, or zone of polarizing activity—and it was assumed that the ZPA contained the morphogen.




**Double trouble.** *A piece of one chick limb bud, transplanted to a second limb bud, causes the growth of extra digits. The chemical culprit may be retinoic acid (inset).*

Later several groups showed that retinoic acid applied directly to the anterior part of the limb bud could mimic the action of the ZPA, inducing formation of the extra digits. That finding made it seem possible that retinoic acid was a morphogen. But in order to be a morphogen, a substance had to be present in the developing embryo in a clearly defined spatial pattern—and no one had ever shown that retinoic acid fit the bill.

Enter Eichele and his colleague Christina Thaller. In 1987 they showed that retinoic acid is not only present in the limb bud but also that it forms a gradient, with the highest concentration in the posterior region containing the ZPA. This finding strongly implicated retinoic acid as the limb bud's native morphogen and stimulated a cascade of later results tying that chemical to the formation of structures throughout the chick embryo and in other species—including newts, frogs, mice, and human beings.

Most of this work has focused on regions that, like the ZPA, are known to provide spatial cues to embryonic tissues. For example, a structure called the floor plate in the chick embryo helps define the direction of neuronal growth in the developing nervous system, as work by Thomas Jessel and his colleagues at Columbia University has shown. Eichele, Jessel, and their co-workers have recently found that the floor plate is able to synthesize retinoic acid. Some of the same workers will soon be examining Hensen's node, which "tells" cells where they lie along the embryo's main anterior-posterior axis so that they can assume appropriate developmental fates.

This preliminary work suggests that retinoic acid may act in many developing tissues. But those results haven't yet nailed down the question of whether retinoic acid is indeed the basic morphogen. Says Eichele: "Hensen's node, the floor plate, and the ZPA share the property of being able to define axial polarities. This does not necessarily mean that these tissues exert their effect through retinoic acid directly. It could be that they make a growth factor in response to retinoic acid. This growth factor—and not retinoic acid—

might be the actual spatial cue."

The most recent work in this hot area is aimed at resolving that question: finding out just how retinoic acid operates and whether its effects are direct or mediated by other substances. A key question is specificity: retinoic acid acts in many tissues—but doesn't have the same effect in all of them. On the contrary, it influences tissues destined to become limbs as well as those fated to become part of the nervous system. "The main problem," says Pierre Chambon of the University Louis Pasteur in Strasbourg, France, "is to explain how such a simple molecule can have such profound and marked effects on so many cell types."

Chambon is not only posing the question—his lab is hard at work looking for answers in the cellular receptors to which retinoic acid binds. Not long ago workers in Chambon's lab and, independently, in the lab of Ron Evans at the Salk Institute identified the first of a family of retinoic acid receptors. Unlike many other cellular receptors, the molecules that bind retinoic acid are not on the cell surface but in the nucleus.

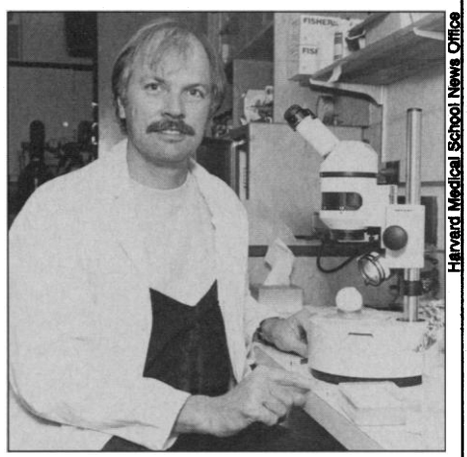
One current paradigm for how retinoic acid works is that it enters the cell passively (without a specific channel) and diffuses to the nucleus. There it encounters and binds to its receptor, a protein whose affinity for DNA increases when it is complexed with retinoic acid. The receptor and its ligand, either alone or with other proteins, regulate the expression of target genes, which in turn determine the final function of the mature cell.

The isolation of the receptor has begun to shed some light on the puzzling question of specificity. In the past 3 years, work in several labs, including Chambon's and Evans', has shown that there is not only one gene for the retinoic acid receptor. In fact, there are at least three, labeled alpha, beta, and gamma, each of which can be processed slightly differently to yield a plethora of receptor variants. "From three genes there are at least ten different receptor molecules, each . . . possibly exerting a specific effect on gene expression," Chambon told *Science*.

In a comprehensive study, Chambon has shown that each of the three receptors has a different spatial pattern of expression in the embryo. The alpha receptor is ubiquitous. The beta receptor is expressed at high levels in the developing nervous system. The gamma receptor is limited to specific tissues, among them cartilage, bone, and skin. With several different receptors available, retinoic acid might thus have quite different effects in different tissues.

But that doesn't fully resolve the problem of where specificity comes from—where the laying down of spatial patterning really begins. How, for example, is a cell cued to express one retinoic acid receptor rather than another—or one combination of receptors rather than another? A tantalizing clue may lie in recent work suggesting that at least one retinoic acid receptor gene is autoregulated: its expression depends on the presence of retinoic acid itself.

And how might autoregulation lead to the required specificity? One theory goes like this. Although the alpha gene is expressed in almost all embryonic cells, only some of those cells are exposed to retinoic acid. When they are exposed, the complex of receptor and retinoic acid will induce expression of other



Harvard Medical School News Office

**Morphogenius.** *Gregor Eichele, in whose lab retinoic acid was implicated as the native morphogen of the embryo.*

retinoic acid receptors and receptor subtypes. The exact constellation of receptor types may be dependent on the amount of complex present in the nucleus, an amount determined by the position of the cell in the overall retinoic acid gradient. And this constellation yields the specific fate of the cell.

As theories like this one suggest, much recent evidence does point to retinoic acid as the fundamental morphogen. But just as things seemed to be coming together nicely, the field was given a good shaking up by the finding that retinoic acid isn't the only retinoid that may be a morphogen.

Earlier this year, Thaller and Eichele demonstrated that didehydroretinoic acid has some of the same properties as retinoic acid, and they believe it, too, is involved in limb development. What is more, the Evans group recently found another class of retinoid receptors (designated RXR) that appear to be distinct from the previously described family of retinoic acid receptors. It is possible that they are activated by a molecule such as the compound identified by Thaller and Eichele.

"This confounds the whole field," says Evans. "Just 1 year ago we had to explain retinoic acid action through three receptors, which was confusing enough. Now we have to account for five or more. Why the dizzying diversity? It seems like a morass, but the physiologic process in the end is beautifully balanced."

Some of that balance may be achieved in a surprising way. Researchers in Evans' lab recently found that the retinoic acid receptor and a completely different type of DNA-binding protein called AP-1 recognize the same stretch of DNA. AP-1 responds to a variety of growth factors and lymphokines, which promote cell proliferation. The same factors are known to inhibit the ability of vitamin A to promote cellular differentiation in bone. According to Evans, the two systems may check one another's activity.

All that is very well, but it doesn't tell us exactly how retinoic acid exerts its effects on the developing cell. For the most part, answers to that question are only now being worked out. Some evidence points to the extracellular matrix, the complex arrangement of polysaccharides and proteins that holds cells together. As work by Chambon's group with Lorraine Gudas of Harvard Medical School suggests, retinoic acid receptors may activate the gene for laminin, a matrix protein. The receptors may also repress the expression of an enzyme that can digest the matrix. The net effect, Chambon says, may be "to modulate the structure of the extracellular matrix, which is important for cell migration and rearrangement in embryogenesis."

In addition, work by Antonio Simeone and co-workers at the International Institute of Genetics and Biophysics in Naples, the Instituto Scientifico H. S. Raffaele in Milan, and the Wistar Institute indicates that retinoic acid can activate a series of human homeotic genes. Homeotic genes, first discovered in the fruit fly *Drosophila*, are known to have a role in spatial pattern formation during embryogenesis.

And that, for the moment, is where the work on retinoic acid in development stands. No doubt in the next year there will be further important advances, including, perhaps, ones that settle the question of whether retinoic acid is indeed the fundamental morphogen or whether its effects are somewhat less direct. Either way, however, some key footholds have been gained. As Eichele notes, describing the initial ZPA work, it is gratifying to extend a "curious duplication experiment into a tremendous heuristic model. We can now propose clear biochemical mechanisms [for embryonic development] and work within a clear intellectual framework." ■ MICHELLE HOFFMAN

*Michelle Hoffman is an editor at the* American Scientist.